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Evaluation of Minerals Content in SolaniumDubium Collected From White Nilein Central Sudan

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Abstract:
Some plants of the family solanaceae such as Solanumdubium"Gubbain", which is a well-known wild plant, grow wildly in many regions in Sudan during the rainy season.Solanumdubiumis used in rural areas for milk coagulation. It is a bushy pubescent herb grown widely in northern, central and western Sudan along with other species such as S. innacum, S. esculentum, S. macrocarpon and S. melongena.Research on S. dubium was focused mainly on obtaining Solanum crude enzyme from the seeds in pure form and commercial production of the enzyme for cheese making. Solanaceous plants are known for their high alkaloidal content in all plant parts including the seeds, which are responsible for their antimicrobial activity in addition to other metabolites such as flavonoids and tannins.
This study was conducted to determine the chemical composition of the seeds ofSolanumdubiumplant and its properties.Plant Solanumdubium have been used as milk coagulants in cheese making for centuries either as crude extracts or in purified form. These coagulants are an alternative to the calf rennet due to the limited availability and high price of rennet, religious factors, diet or ban on recombinant calf rennet in some countries. These enzymes are found in almost all kinds of plant tissues and can be obtained from their natural source or through in vitro culture to ensure a continuous supply of plant proteases. The excessive proteolytic nature of most plant coagulants haslimited their use in cheese manufacturing due to lower yields of cheese, bitter flavors and texture defects. The search for new potential milk-clotting enzymes from plants still continues in order to meet the increasing global demand for diversified and good quality cheese production. For the great important uses ofsolanum,the study aim's to focus of the active chemicals component in solanum seeds.
The seeds of Solanumdubium were blended and extracted using different types of buffers. The most reliable, quick, and efficient buffer was found to be 5% NaCl in acetate buffer (pH 5.0) which was used throughout the study. The extract was filtered and fractionated twice with ammonium sulphate. When compared with other plant enzymes, S. dubium enzyme was found to have higher clotting and proteolytic activities. The activity of the enzyme was steadily increased with enzyme and substrate concentration. The enzyme was found to be very stable against a wide range of pH values as well as a wide range of temperature (20–90 °C). The results of substrate specificity of the enzyme showed that the partially purified enzyme preferred both hydrophilic and hydrophobic amino acid residues at P1 position. The catalytic efficiency of the purified enzyme was enhanced by an aliphatic amino acids (Leu) compared to aromatic residue (Phe) at P1 position at the same site.And the morphology of solanum seeds was studied with scanning electron microscopy of mucilage at different magnification using Philips, Lancashire, XL-30 SEM and the results show in figures 5, 6, 7). Also the fractionation of structure was observed with infera red spectroscopy in figure (8).
In this study some metals was determined like Mn , Cr, Cd , Zn. , Fe, and basic element like Ca ,Mg , K , P in solanum seeds , the sample observed that maximum permitted levels is K higher concentration one and other metal flow respectively Ca, Cr, Fe, Cd , Zn, and P .Seed samples of solanumcollected fromKenana area in white Nile . Metal concentrations found in solanum seeds are shown in Table 2 and Table 3. The study showed significant positive correlation metal content in solanum contain including heavy metals.The results showed that all chemical components under study were not significantly affected by the type of coagulant insolanum uses in cheeseindustry.
Keywords*:plant Solanumdubium , (Gubbien) seed, chemical element , enzymedubumin ,solventextraction.*

I. Introduction

Solanum is a large and diverse genus of flowering plants, which include two food crops of high economic importance, the potato and the tomato. It also contains the nightshades and horse nettles, as well as numerous plants cultivated for their ornamental flowers and fruit.

Solanum species show a wide range of growing habits, such as annual and perennials, vines, subshrubs, shrubs, and small trees. Many formerly independent genera like *Lycopersicon* (the tomatoes) and *Cyphomandra* are now included in *Solanum* as subgenera or sections. Thus, the genus today contains roughly 1,500–2,000 species (20, 17,28).

Fig. 1. Solanumdubium whole plant (left), fruits (yellow and green) and seeds (black clusters)

Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Asterids
Order:	Solanales
Family:	Solanaceae
Genus:	*Solanum*
Species:	***S. dubium***

Binomial name*Solanumdubium*

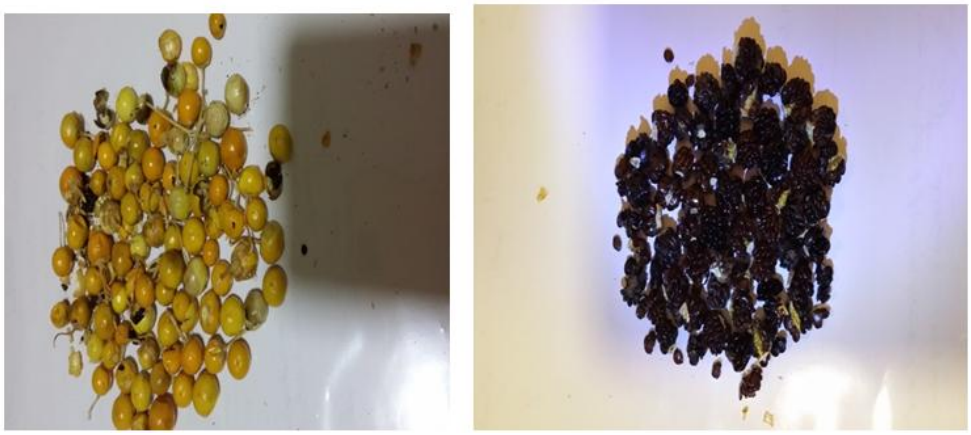


Fig. 3. The fruits with coat and see of Solanumdubium

The genus *Solanum* consists of over 2000 species distributed worldwide is the largest member of the Solanaceae and is one of the largest genera among all flowering plants (19). The species of the family Solanaceae are medicinal herbs (6), and contain unique alkaloids and other biochemical constituents used for the treatment of diverse ailments such as diabetes, cholera, bronchitis, high blood pressure and as laxatives (8).

1.1 Plant and uses

Solanumdubiumfresen is an indigenous plant wildly grown in central, northern and western Sudan, it is a woody herb, unripe fruits are greencolor, while the ripe ones are yellow, and fruits usually dry on the stem. The seed is dark brown in color, and due to its bitter taste animals do not eat it. Dairy farmers in some parts of the Sudan use the berries of *Solanumdubium*to make white soft cheese known as *GibnaBayda*from goat and sheep milk (29, 28).

The resultant cheese has a slight bitter taste and a fragile crumbly texture, and the bitterness being caused by the presence of some alkaloids or nonspecific proteolytic activity of enzymes that is obtained from the berries of *Solanumdubium*(3). No attempt has been made in Sudan to investigate the production of *Mudaffara*cheese from plant enzyme (1, 2,4).

It animal rennet is not available or slaughter od calves for chymosin is not feasible or the cheese is only for vegetarians, vegetable rennet becomes very important and the use of vegetable rennet for cheese making could contribute to improving the nutrition of those populations whereas restriction are imposed against the use of animal rennet (2,6,8,15,19) .

Plant source for milk clotting enzymes had been identified from different plants including *Solanumdubium*. *Solanumdubium*is a well-known species belonging to the family Solanaceae (Suleiman et al.

1988). It is also described as a bushy pubescent herb plant distributed in different regions in Sudan. The *S. dubium* fruit was found to have a high concentration of rennin-like compound (9) that has been used for white cheese production purposes. Some research showed positive results using *Solanumtorvum*and *Solanum dubium* for the manufacture of white cheese (3,5,4,20)

Dawla (2001) found that cheese produced using rennin extracted from *S. dubium* is characterized by its light, soft and compact texture, preferentially better than that produced using commercial rennin materials. Recently, much research interest has been directed towards discovering a milk-clotting enzyme from natural plants that could satisfactorily replace commercial rennet in cheese making (14). In general, callus (unorganized aggregate of cells) can be induced from an excised part of a plant (explants) in which callus growth after culturing of the explants can be continued and regeneration of whole plants (plantlets) can be readily obtainable

Their research showed positive results in using this extract for manufacture of Sudanese white soft cheese. This research aims to study the chemicals component and their characterization of *Solanumdubium*seeds (5, 9, 16, 27).

1.2 Solanumdubiumanantioxidants:

In the light of recent scientific developments, the medicinal properties of plants have been investigated, throughout the world due to their potent pharmacological activities and economic viability. A great number of aromatic and medicinal plants contain compounds, exhibiting antioxidant property. The natural antioxidants are primarily plant phenolic compounds that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks **(**Pratt, 1990**)**. Many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, anticarcinogenic, antibacterial or antiviral activities to a greater or lesser extent **(**Sala *et al*, 2002**).** Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolicare increasingly of interest in food industry, because they retard oxidative degradation of lipids and thereby improve the quality and nutritive value of food **(**Kahkonen*et al*, 1999**;** Rice *et al*, 1995**).**

Solanumdubium can mixture with honey to raise the activity for uses in industry of cheese and medicine .The curative potential of honey is well documented in the oldest medical literatures and religious testaments. At the research level, honey is currently showing potential in minimizing cellular injuries of the skin and post-radiotherapies (21, 9, 16).

II. Materials And Methods

2.1 Sample **of *Solanumdubium*plant**

The plants were collected from Kenana area,Wight Nile in central of Sudan between December 2016 and January 2017. The coats and seeds were separatedand carefully cleaned, washed several times withdistilled water and the coats were then coarselypowdered using electric grinder. The coats wereanalyzed to determine dry matter, ether extract, crudeprotein, crude fiber and ash. The cleaned and shade-dried plant seeds were powdered using a grinding machine; each ground sample was weighed and stored in a dry container at ambient temperature.

And the seed powder after crushed was analyzed to determinetheir chemical components.

2.2. Preparation of samples:

All samples were treated chemically to allow measuring of concentrations of Ca, Fe, Zn, P, Cd, Mn, Cr,K, and Mgcontent in the parts of plant using different instruments. The parts of solanum plant were dried and grinded softly in a ceramic mortar and sieved. The powdered kept in bottles to use in next steps.

2.2.1. Sample preparation for AAS:

The parts of solanum plant were dried and grinded softly in a ceramic mortar and sieved. 0.5g of the sample was placed into 100 ml Teflon beaker, 6 ml of (1:5) per chloric acid and nitric acid were added and allowed to stand at room temperature overnight for initial the reaction , and subsequently 10 ml of concentration $HClO_4$ were added to the mixture .The Teflon beaker was tightly stopping (to reflux the vapours of the acid) and heated on sand bath at200-250 c for at least 6 hours until complete digestion was achieved , which is indicated by anon – turbid and/or a white solution . Then the solution was evaporated to dryness or semi dryness. The residue was diluted with distilled water into 50 ml volumetric flask. The prepared solution was placed in to 50 ml of polyethylene bottle and stored at room temperature to be ready to use for AAS within 30 days.

2.2.2 Preparation *Solanumdubium*seed extract

Ten grams of the crushed seed were shaken with100 ml distilled water and then the mixture wasblended by agitator for 15 min until the mixturebecame homogeneous. Seed extract was placed in therefrigerator at 4ºC for 24 hr, filtered and 20 ml of theextract was used for analysis.

2.3 Scan electron microscopy (SEM) of solanumdubium seeds:-

Solanumdubium samples were placed on brass analysis stubs and placed into a JEOL JSM-6380LV SEM. The samples were run as received without any coating or processing. The microscopy power was kept low (to prevent imaging problems) and images were taken at varying magnifications under vacuum (figures 5, 6 and 7).

2.4Fourier transform analysis

Fourier Transform Analysis (FTIR) spectra of mucilage were recorded on a FT-IR spectrometer (Thermo Scientific). The dry powder was mixed with KBr and pressed into pellets under mechanical pressure. The FT-IR spectra were obtained by scanning between 4000 and 500/cm.

2.5 Extraction of Solanum dubium seed :

The crude enzyme was extracted by the following methods:-

Freezing and evaporating under reduced pressure (freezing drying):-

Coarsely powdered yellow fruits and seeds (100g each) were macerated in conical flask for 24 hour using distilled water with occasional shaking for the first three hours and solutions were then filtered .The filtrate was spread on shallow basin surrounded by afreezing mixture under vacuum for water evaporation within two hours (13, 25, 10) .

2.5.1 Extraction with distilled water:-

Five grams of the crushed material were shacken with 30 ml distilled water for 15 minutes at room temerature and then filtered . The aqueous filtrate was used for determined the enzyme activity and concentration(22,25) .

2.5.2 Preparation of 96% ethanol, methanol and water extracts

Five hundred grams of sample, *S. dubium* seeds combination was extracted by maceration with 96 % ethanol using shaker apparatus. Extraction was carried out for three days with daily filtration. The filtrates were combined and the solvent was evaporated under reduced pressure using a rotary evaporator. The same procedure was repeated with methanol (10, 13, and 21).

III. Results and Discussion

The present study was conducted to investigate the enzyme extract and to identify metals, content in Sudanese *S. dubium* seed (27).

The chemical composition of *Solanum dubium* coat was as follows:

crude fiber 57.9%, crude protein 12%, dry matter 97.05%, ash 8.5%, nitrogenfree extract 10.89%, fat 5.78%. The chemical composition of black cumin was as follows: crude fiber 27.25%, crude protein dry matter 95.32%, ash 3.40%, fat 19.12%, nitrogen free extract 15.30%.

Table(1) Substrate specificity of Solanum dubium enzyme on different peptide substrates**.**

Substrate	**substrate concentration (mM) Km $_{(mM)}$**	**Kcat$_{(s^{-1})}$**	**Kcat/Km $_{(M^{-1}s^{-1})}$**
			$4.89 \_ 10^{+4}$
N-Suc-Ala-Ala-Pro-	2.0–0.26	1.54 6.8 - 10	
Phe-pNA			$1.9 - 10^{+2}$ $3.96 \_ 10^{+4}$
N-Suc-Ala-Ala-Pro-	2.0–0.26	5.02	
Leu-pNA			$4.2 - 10^{+2}$ 3.08_ 10+5
Ac-Tyr-Val-Ala-	1.01 – 0.25	1.40	
AsppNA			
N-Suc-Ala-Ala	2.0 – 0.24	0	0 0
AlapNA Figure 5			

3.1 Scan electron microscopy (SEM) :-

Scanning electron microphotographs (SEM) of mucilage obtained is represented in Figure 5, 6, 7 at different magnifications. The microphotographs of mucilages are indicative of an amorphous material. The particles are mostly seen as aggregates of irregular shapes and dimensions which were fibrous in nature. The SEM results of the present study suggest that, hydration capacity of mucilage depends on the surface property. The shape and structure or surface topography of the mucilage may be affected by the method of extraction and purification or preparation of the products reported that, particle size and specific surface area influence the hydration behavior of solanum, which in turn influence their intrinsic and molecular mass. They also reported that particle size influenced the hydration kinetics and molecular mass of solanum dubium.

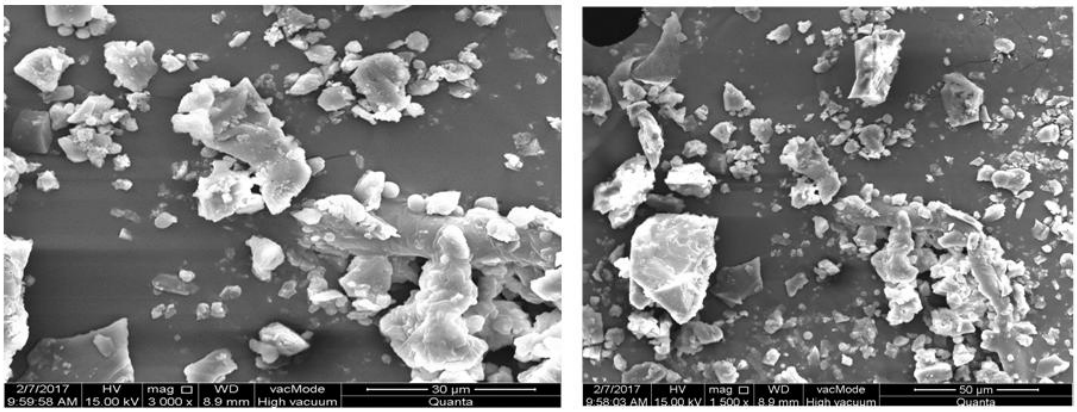


Figure (5) and (6)SEM of solanum seeds in high vacuum

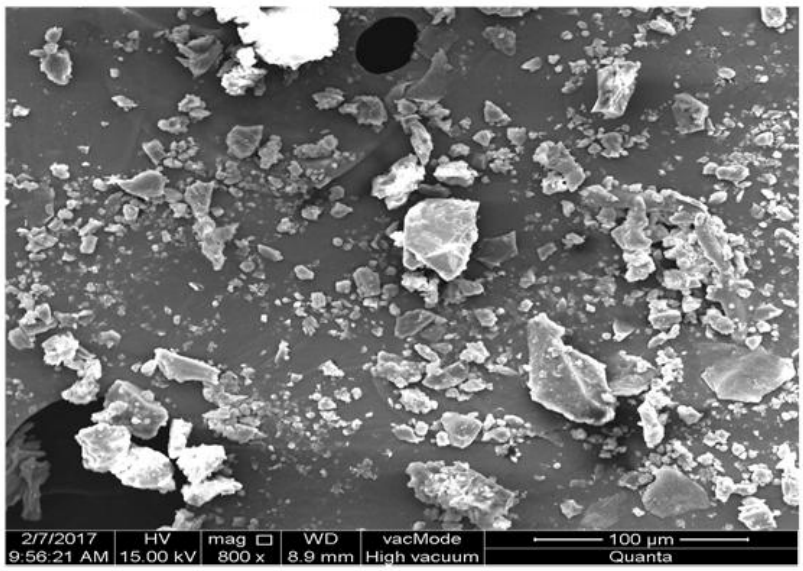


Figure 7-solanum seedsScanning electron microscopy of mucilage at different magnification using Philips, Lancashire, XL-30 SEM.

3.2 Infera red spectroscopy of solanum dubium

Fourier transform analysissoftware was used to interpret. Spectra exhibited the typical bands and peak characteristic of solanum. The spectra of mucilage shows band occurring at 2924.06 cm^{-1}-results from the presence of 2809 cm^{-1} results from stretching modes of the alkyl C-H stretch or CH_2, carboxylic acid C= FTIR spectrometry has been extensively applied to characterize the polymer's molecular and material structure. Characterization using FTIR spectroscopy often results in the identification of functional groups and the modes of their attachment to polymer backbone (Baxter *et al.,* 1992). The FTIR spectra exhibit the typical bands and peak characteristic for mucilage. The FT-IR spectrum of mucilage is presented in Figure 8. 'CHEMIX' School O methylene (-CH_2-) C-H stretch. The peak obtained at 1635.24 cm^{-1} results from stretching mode of the alkenyl C=N and C=C stretch. Absorption bands around 1635.24 and 1379.30 $cm^{-1}$is CH3 or C-H methyl rock .Also absorption peaks at 1745.14 cm^{-1}result of C=O. The band 1160.80 is CO or S=O sterch. The band 720.16 CH_2rocking and 563.50 band is C=C.

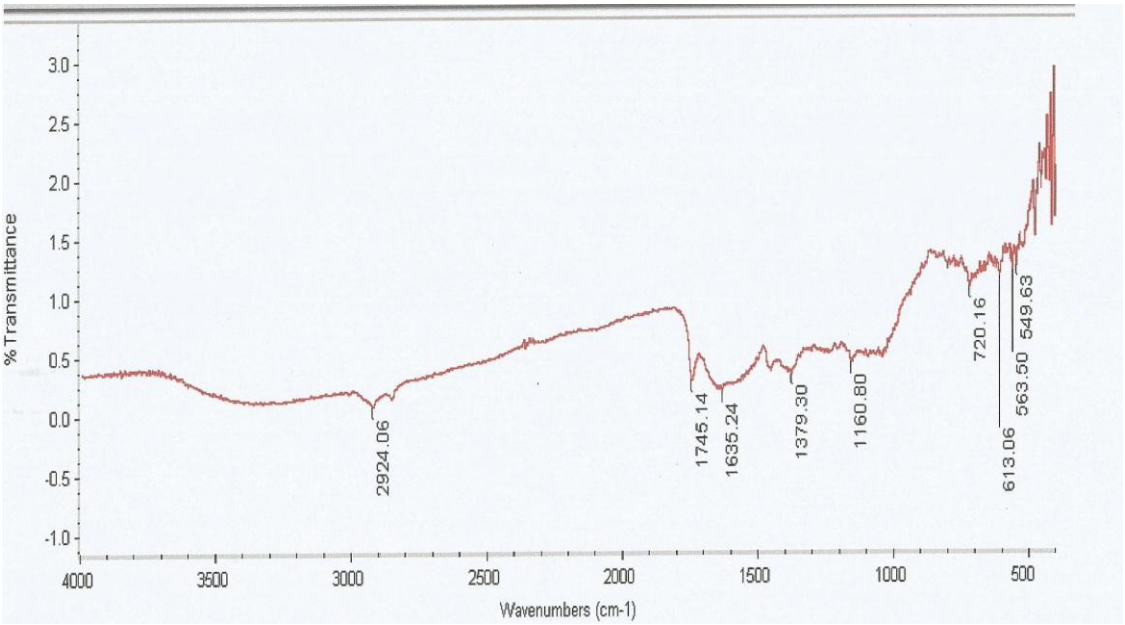


Figure (8)infera red spectroscopy of solanum dubium

3-3 results ofmetals analysis:

Metal content in tobacco depends on soil properties, atmospheric conditions, and requirements for solanum farming (useof pesticide and fertilizer). Solanum plants take up Potassium and Calcium from soil and concentrate these metals in leaves. Forthis reason, there are large variations in the content of metals in tobacco between countries.

In this study content of Mn , Cr, Cd , Zn. , Fe, and basic element like Ca ,Mg , K , Pin solanum seedswas determined, the sample observed that maximum permittedlevels its K it higher concentration one and other metal flow respectively Ca, Cr, Fe, Cd , Zn, andP,but all of them are found higher concentration in solanum seed samples collected from Kenana area in Wight Nile. Metalconcentrations found in solanum seeds are shown in Table 2 and Table 3 The study showed significant positivecorrelation metal content in solanum contain including heavy metals .

A significant positive correlation was observed between metals content in solanum. These results suggest the possibilitythat the higher concentration of metals in solanum refer to the origin from source.

Table (2)concentration of metals in solanum dubium

Element	Element Symbol	Wavelength(nm)	Lamp Current Low (mA)	Slit Width (nm)
Calcium	Ca	422.7	10	0.5
Magnesium	Mg	285.2	8	0.5
Manganese	Mn	279.5	10	0.2
Chromium	Cr	357.9	10	0.5
Cadmium	Cd	228.8	8	I.0
Zinc	Zn	213.9	8	0.5
Ferric	Fe	248.3	12	0.2
Potassium	K	582.0 (ppm)	-	-
Phosphorus	P	12.0 (ppm)	-	-

Table (3) Atomizer / Gas Flow Rate Setup

Element	Element Symbol	Fuel gas flow rate(L/min)	Flam type	Burner Height(mm)	Burner lateral pos.(pulse)	Burner angle (degree)
Calcium	Ca	2.0	Air- C_2H_2	7	0	0
Magnesium	Mg	1.8	Air- C_2H_2	7	0	0
Manganese	Mn	2.0	Air- C_2H_2	7	0	0
Chromium	Cr	2,8	Air- C_2H_2	9	0	0
Cadmium	Cd	1.8	Air- C_2H_2	7	0	0
Zinc	Zn	2.0	Air- C_2H_2	7	0	0
Ferric	Fe	2.2	Air- C_2H_2	9	0	0

Table (4) Measurement Parameters

Element	Element Symbol	order	Zero Intercept	Conc . Unit	Repetition Sequence	Pre-spray time(sec)	Integration time(sec)	Response time
Calcium	Ca	1 st	No	Mg/L	SM-M-M	3	5	1
Magnesium	Mg	1 st	No	Mg/L	SM-SM	3	5	1
Manganese	Mn	1 st	No	Mg/L	SM-SM	3	5	1
Chromium	Cr	1 st	No	Mg/L	SM- SM	3	5	1
Cadmium	Cd	1 st	No	Mg/L	SM-SM	3	5	1
Zinc	Zn	1 st	No	None	SM- SM-	3	5	1
Ferric	Fe	1 st	No	None	SM-M-M	3	5	1

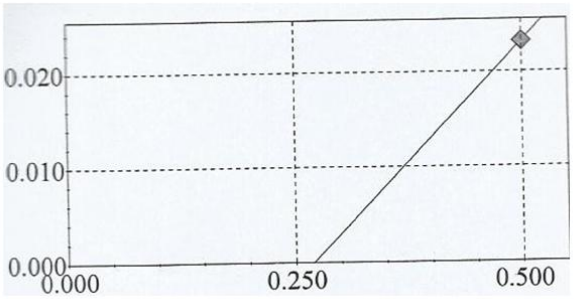
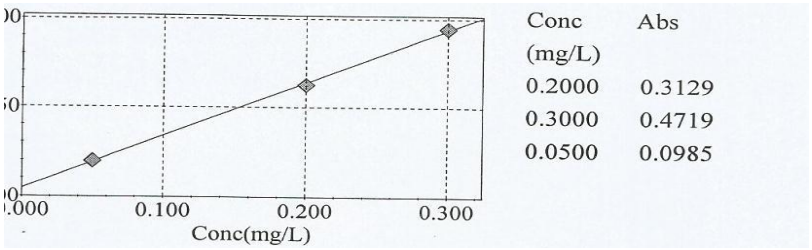
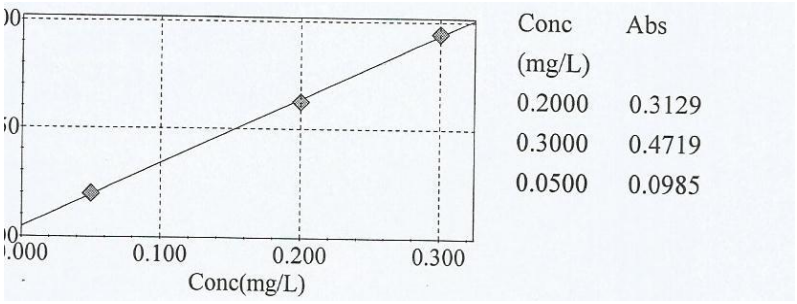


Figure (9)calibration curve of calcium concentration in solanum dubium



Abs =1.48853 conc +0.0215368 , r = 0.9996

Figure (10) calibration curve of magnesium concentration in solanum dubium



Abs =0.933595 conc +0.00472671, r = 0.9992

Figure (11) calibration curve of manganese concentration in solanum dubium

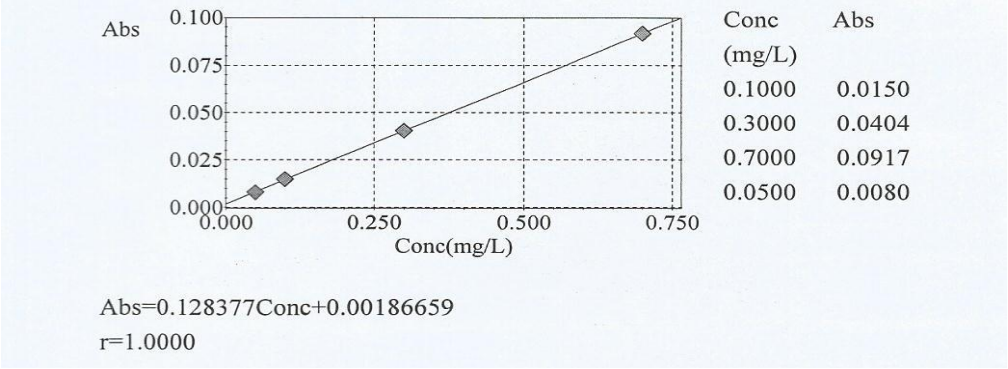


Figure (12) calibration curve of cadmium concentration in solanum dubium

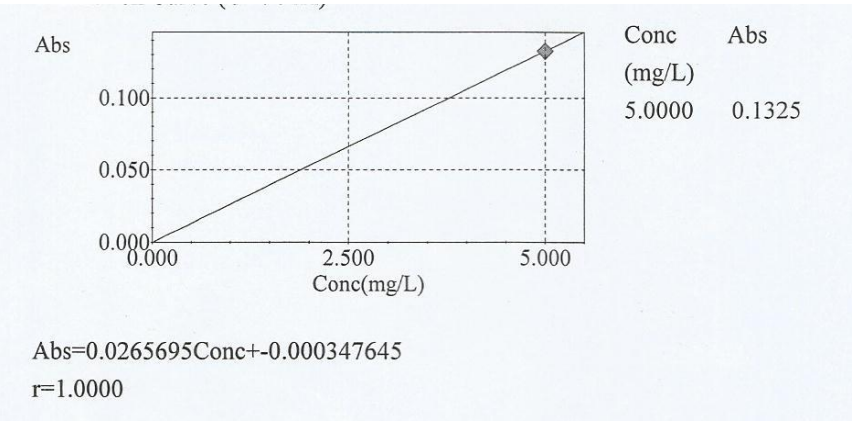


Figure (13) calibration curve of chromium concentration in solanum dubium

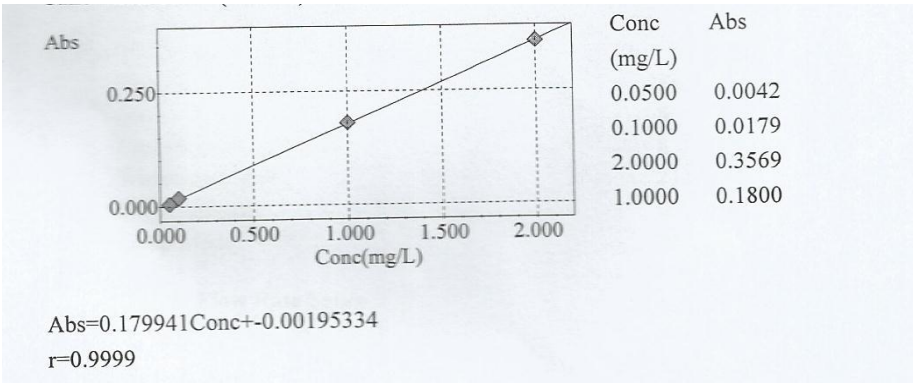


Figure (14) calibration curve of zinc concentration in solanum dubium

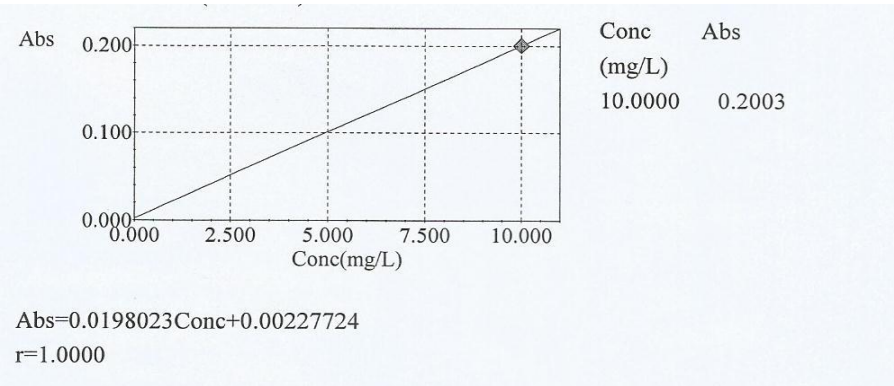


Figure (15) calibration curve of ferric concentration in solanum dubium

3.4 Effect of pH and temperature on the activity and stability of the purified enzyme.

(a) PH stability of the purified enzyme. The enzyme (12μg) was incubated atvarious pH values for 1 or 25 h at 37°C. Residual activity was measured after 40min at 30°C and pH 8.0. (b) Effects of pH on the activity of the purified protease (14).Enzyme activity was determined using 1% azocasein as substrate at variousranges of pH 5.0–12.8 at 30°C for 30 min. The buffer used for pH 5.0- 6.0 was 50mM acetate/ Phosphate, for pH 6.0–8.0 it was 50 mM Tris-HCl, and for pH 8.0–12.5 it was 50 mM glycine-NaOH.

3.5 Effect of pH on partially purified enzyme activity

As shown in Fig (16), the purified enzyme is stable under a wide range of pH and it retained all of its activity in the pH range from dubium seeds for milk-clotting. However, further purification is required to get a highly pure enzyme (11, 14).

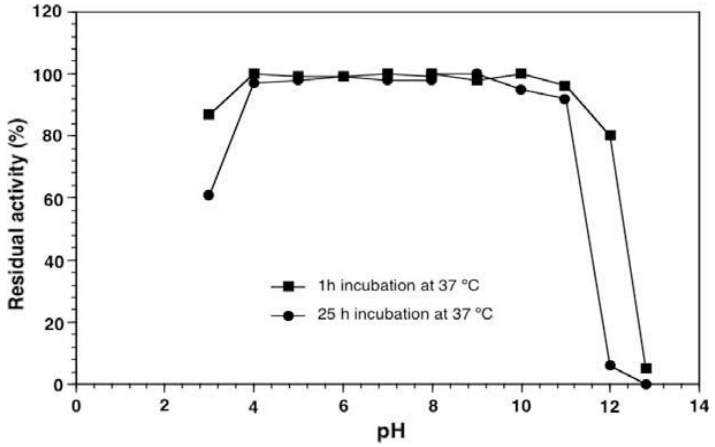


Figure (16)Effect of pH on the stability of partially purified enzyme from Solanumdubium seeds.

.6. Effect of Temperature on partially purified enzyme activity

The results obtained in Fig. 16 showed that the enzyme activity increased as the temperature increased from 20 to 70 _C. The activity at 70_C was 5- and 10-fold higher than that of the activity at 40 and 20 _C, respectively. The activity rapidly decreased as the temperature raised over 80 _C. Since the enzyme had a high optimumtemperature, its stability at a temperature ranged from 20 to 90 _C was studied. There was a total retention of activity after 1 h incubation at 60 _C and about 70% of its activity was retained at 70 _C when the enzyme was incubated for 1 h. The thermo stability of the enzyme was found to be up to 70 _C. The temperature profile of the enzyme was agreed with those of subtilisin/cucumisin likeplant serine proteases reported by Asif-Ullah et al. (2006), Yamagataet al. (1989), and Uchikoba et al. (1990).

In conclusion, compared to other purification procedures done we concluded that a simple purification procedure has been developed in this study to obtain a very active and stable enzyme from S. dubium seeds for milk-clotting. However, further purification is requiredto get a highly pure enzyme (11, 14, 27).

Enzyme activity and stability

Coagulants should not be sensitive to variations in milk composition and pH, sincethe use of highly pH-sensitive rennet can lead to reduced yields and defective cheese due to soft coagulum at cutting (Harboe and Budtz, 1999). *S. dubium* milk-clotting enzyme is stable under wide range of pH (4.0~11.0), and act optimally at pH11.0 (Fig17). The isolated enzyme is more stable at basic pH, and its stability is more comparable to those of Cucumisin-like serine proteases from *Cucumis trigonus* Roxburghi, *Cucumis melo* L. var. Prince, *Euphorbia milii*, and *trichosantuskirrilowi*A (Asif-Ullah *et al.*, 2005; Yamagata*et al.*, 1989; Yadav *et al.*, 2006; and Uchikoba*et al.*, 1990), respectively. These characteristics are important, because most enzymes are catalytically unstable at alkaline pH values, thus limiting their usefulness as cheese making coagulants (Lamas *et al.*,2001) (20,27).

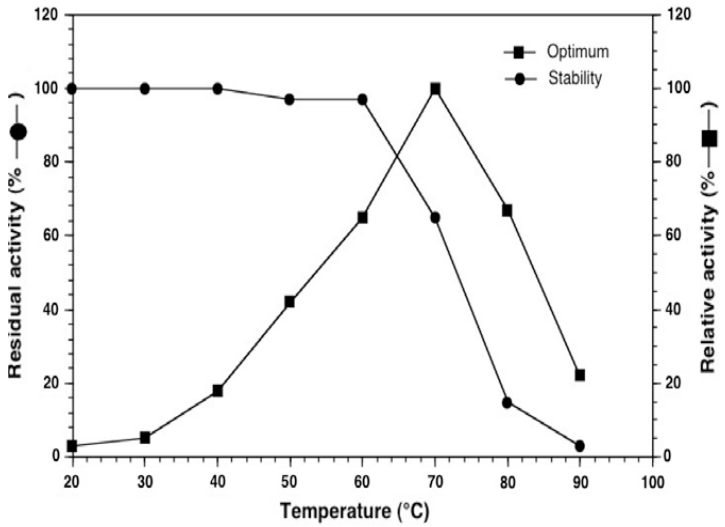


Figure (17) .Effect of temperature on the activity and stability of partially purified enzyme from Solanumdubiumseeds

IV. Conclusions

The study show that the content of mineralsMn , Cr, Cd , Zn. , Fe, and basic element like Ca ,Mg , K , P in solanum seeds , the sample observed that the maximum permitted levels is K higher concentration one and other metal flow respectively Ca, Cr, Fe, Cd , Zn, and P but all of them are found higher concentration in solanum. A seed sample of solanum was collected from Kenana area in Wight Nile. Metal concentrations found in solanum seeds are shown in Table 2 and Table 3 The study showed significant positive correlation metal content in solanum contain including heavy metals .

The results illustrate that all chemical components under study were not significantly affected by the type of coagulant in solanum uses in cheese industry. But must be care by take small weight when we using the solanium seeds powder in cheese industry. 'The extraction of the enzyme by distilled water produced higher activity, and increase in soaking time reduced the activity. As the fruit changes to dry yellow the activity increases. While milk temperature (up to 80°C) increases activity, preheating temperature (up to 80°C) decreases the activity, and the incubation temperature (up to 80°C) and volume of extract (up to 1 ml) increases the activity.

And the enzyme extracted from solanum is good alternative enzyme and cheap for peoples can use it in all kind of cheeses industry.The researcher recommended continuing the research insolanumdubium to determine the other active compounds and to developing the methods and technic for purification and separation the seed from coat.Also to discovery the structure of solanumdubiuminenzyme.

References

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